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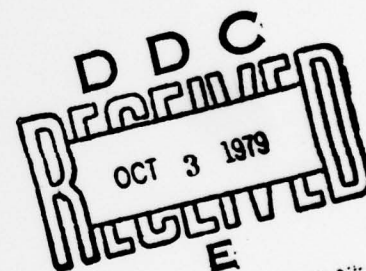Contract #N00014-78-C-0610

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TECHNICAL REPORT NUMBER 1

The Physical and Functional Analysis of Interferons

by

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Introduction

Interferons are glycoproteins which induce states of cellular resistance to viral multiplication. It is now evident that interferons are capable of other biological actions such as modulation of the immune response (both humoral and cell-mediated) (1,2), inhibition of cell division (3), and suppression of intracellular multiplication of organisms other than viruses (4,5). Interferons produced by different cell types can differ in their physicochemical, biological, and antigenic properties. Three classes of interferons are recognized: leukocyte (generally made by cells of lymphoid origin), fibroblast (generally produced by cells of non-lymphoid origin) and "immune" or type II interferons (produced by sensitized T-lymphocytes after exposure to the specific sensitizing antigen). The existence of multiple forms of interferon suggests that each type may have been adapted through evolutionary pressures to perform a particular in vivo function.

The immediate goal of our research is to analyze each of the different classes of interferons with regards to their physiochemical, antigenic, and biological properties. Possible structure to function relationships of each interferon are being examined through the use of specific anti-interferon sera to assess the degree of neutralization of different interferon activities in a recently developed quantitative interferon neutralization assay. The resulting antibody neutralization curves for each interferon activity under study reflects the relative amount of interferon required for the expression of that activity, and differences in the slopes of the curves would indicate different molecular mechanisms involved in the expression of different activities. The results of these studies should reveal whether the various biological activities attributed to interferons are mediated through a single site or multiple molecular sites on the interferon molecule.

In the past year, we have been studying the mechanisms by which interferons interact with cells of homologous and heterologous species to induce antiviral resistance. The results of our studies of the antiviral activities of human interferons on bovine and murine cells have demonstrated that, the interaction of human fibroblast interferon with bovine cells results in only a partial development of the antiviral state, and it is totally inactive on murine cultures. On the other hand, treatment of bovine and murine cells with human leukocyte interferon results in the full development of antiviral resistance, and the resulting dose-response curves resemble those observed for human cells.

We have also found that the ability of various murine interferons to protect both human and bovine cells is due to a subspecies of murine interferon which is antigenically related to human leukocyte interferon. Moreover at the time of this writing, we have identified two distinct bovine interferons which are active on human cells. One of these bovine interferons possesses a common antigenic determinant(s) with human leukocyte interferon. Collectively, these findings indicate that cells from phylogenetically diverse species can synthesize a class of interferon molecules which are structurally similar. This implies that the gene for leukocyte interferon has been rigidly conserved during evolution.

There is evidence which suggests possible immunomodulatory activities for interferons both in humoral and cellular immunity. Investigators have reported, that depending on the time and quantity of interferon administered, interferons can either enhance or depress the antibody response to a number of antigens (1). Other studies have shown that B- and T-lymphocytes release interferons during specific mitogenic stimulations (1,6). The T-lymphocyte plays a central role in cell-mediated immunity, and during specific, mitogen-stimulated replication of these cells a number of soluble mediators (lymphokines) are released from them. One of these lymphokines has been found capable of inducing antiviral resistance in cell cultures, and has been referred to as "immune" interferon (7). The immune interferon differs from virus-induced interferons in the physical properties originally used to characterize interferons and for this reason it has been classified a type II interferon. Potent neutralizing antisera specific for human leukocyte or fibroblast interferons do not neutralize the antiviral activity of human type II interferon. A just published report (8) has shown that type II interferon is relatively more active in suppressing cell division than type I interferons and it is possible that the primary function of type II interferon is in the suppression of cell division of other cellular elements involved in immunity and its antiviral activity may prove to be a secondary property. One aim of our research is to fully characterize this interferon as to its physicochemical and biological properties.

Methods

Cells. The diploid FS-4 skin fibroblast cell strain used in these studies was originally derived from a single neonate foreskin in the laboratory of Dr. Jan Vilcek, New York University. A strain of human skin fibroblast cells trisomic for chromosome 21, designated GM 2504, was obtained from the Mammalian Genetic Mutant Cell Repository (Camden, N.J.). A line of bovine kidney cells (MDBK) was provided by Dr. P. Sehgal (Rockefeller University). The VERO line of African Green monkey kidney cells used for virus plaquing was the kind gift of Dr. Jan Vilcek, New York University. All cells were propagated in Eagle's minimal essential medium (MEM) supplemented with 5% fetal bovine serum.

Interferons and anti-interferon sera. Human fibroblast (F) interferon was prepared by inducing FS-4 skin fibroblasts by the superinduction method of Havell and Vilcek (9). Human leukocyte (LE) interferon was prepared by Dr. K. Cantell (Helsinki, Finland). Interferon assays were done by the micro-titer method, Armstrong (10) as modified by Havell and Vilcek (9). The titer of the human fibroblast standard (G023-902-527) was found to be its assigned value when titrated on diploid skin fibroblasts and 10 times greater when assayed on GM2504 cultures. The interferon units reported in the text are standard reference units/ml based on interferon assays done on normal diploid skin fibroblasts.

Antisera. Rabbit anti-human F serum was prepared as described earlier (11) using purified F interferon ($>10^7$ units/mg protein) as the immunogen. Sheep anti-Le serum was the kind gift of Dr. L. Borecky (Bratislava, Czechoslovakia).

Interferon titrations and antibody neutralizations. Human Le and F dose response titrations were done on confluent homologous human and heterologous bovine cultures grown in Falcon (Oxnard, Ca.) 3008 multiwell tissue culture plates. After an 18 hour incubation period, the interferon was removed and the cells were inoculated with vesicular stomatitis virus (VSV) (Indiana type) at a multiplicity of infection (MOI) of 1. One hr after the adsorption period, the cultures were washed 3 times with Hank's balanced salt solution and were reincubated. Nine hours later the VSV was harvested and titrated by plaquing on Vero cells. The yields of virus are presented as $\log_{10}$ inhibition of VSV/ml which were determined by subtracting the $\log_{10}$ virus yield of an interferon treated culture from that of control cultures.

Interferon neutralization assays were done by reacting a constant quantity of each interferon (20 international reference units/ml) in an equal volume of serial four-fold

dilutions of antisera. These mixtures were then incubated at 37°C for 1 hr and then placed on the cell cultures and assayed for residual interferon activities as in the interferon dose response assays by means of VSV inhibition.

Results

I. Analysis of the Antiviral Activities of Human Leukocyte
    and Fibroblast Interferons on Cells of Different Species.

Initially, interferons were characterized as "species
specific", because interferons produced by one particular
species were found to be active only on cells of that species.
However, as more potent preparations of interferons became
available it was found that some interferons were capable
of limited activity on cells of certain species. Human leu-
kocyte interferon, for instance, was reported to be very
active on porcine and bovine cells, whereas human fibroblast
interferon was shown to be considerably less active on these
cells (12). Thus, the degree of antiviral activity on cells
of heterologous species has proven a useful parameter for dif-
ferentiating interferons. Several laboratories have also re-
ported that human fibroblast and leukocyte interferons differ
in their ability to induce antiviral activity on human cells,
in that fibroblast interferon was more effective than leukocyte
interferon in inducing antiviral resistance on fibroblast
cells (11). In comparing the rates of induction of the anti-
viral states of human fibroblast cells by the three distinct
human interferons, it was found that human fibroblast inter-
feron induced the greatest degree of resistance in the shortest
period of time. Type II interferon required a considerably
longer period of time to induce antiviral resistance than
leukocyte interferon (F. Volvowitz, personal communication).
These findings indicate that the different interferons interact
differently with different cells, and that tissue specificities
may exist for the different interferons.

Little information is available concerning the initial
interaction of interferon with the cell and of the events
that result in the induction of the cellular antiviral state.
It is thought that the first step in the induction of the anti-
viral state involves the binding of the interferon molecule to
a receptor molecule located on the external membrane of the
cell (13,14). The appropriate interferon-receptor interaction
results in the triggering event that invokes the development
of viral resistance. Since there are distinct classes of
interferon which differ in their relative antiviral activities
on both homologous (11) and heterologous (12) cells, these
differences in antiviral activities probably reflect different
interactions of the interferons with the cellular receptors on
each cell type.

In the past year, we have been comparing the antiviral activities of human leukocyte and fibroblast interferons on cells of different species by means of interferon dose-response studies. In figure 1 are presented the results of a log dose-response study comparing the antiviral activities of human leukocyte interferon on human, bovine, and murine cell cultures. The cell cultures were treated with designated quantities of human leukocyte interferon (units/ml) for 18 hours. After this the cells were infected with vesicular stomatitis virus (VSV) at a multiplicity of infection (MOI) of 1, the virus was harvested 10 hours later, after a single cycle of multiplication, and the samples titrated by plaquing on Vero cells. It can be seen that the resulting log dose-response curves for human leukocyte interferon on all three types of cells are regularly sigmoidal, and that the slopes are all approximately the same. The similar slopes of the dose-response curves would suggest that the mechanism by which leukocyte interferon induces antiviral resistance on all three cells is the same. The distance between the curves relfect the relative sensitivity of each cell type to the antiviral action of human leukocyte interferon. The differences in sensitivities of the different cells is probably an expression of the relative affinity of the leukocyte interferon for each cell's interferon receptor sites.

In a similar log dose-response study using human fibroblast interferon it was observed that while the resulting curve of virus inhibition was sigmoidal on human cultures, the dose-response curve on the bovine cells was dramatically different (Figure 2). The curve of inhibition on bovine cells exhibited a plateau at only 1.5 logs of virus inhibition at all fibroblast interferon concentrations tested up to 500 interferon units. In an independent study we have found that a preparation of human fibroblast interferon titering 10^5 units is inactive on murine cultures.

The results of these studies reveal that the action of human leukocyte and fibroblast interferons on cells of other species are clearly different. Human fibroblast interferon induces only a partial state of antiviral resistance on bovine cultures at concentrations up to 500 interferon units, and is inactive on murine cells, whereas the dose-response curve for human leukocyte interferon resembles the sigmoidal curves obtained on human cells and on murine cells. These observations demonstrate that human fibroblast interferon is restricted in its ability to cross the species barrier. This restricted antiviral activity could reflect an incomplete interaction of the fibroblast interferon with the bovine receptor or alternatively it could mean that fibroblast interferon may bind
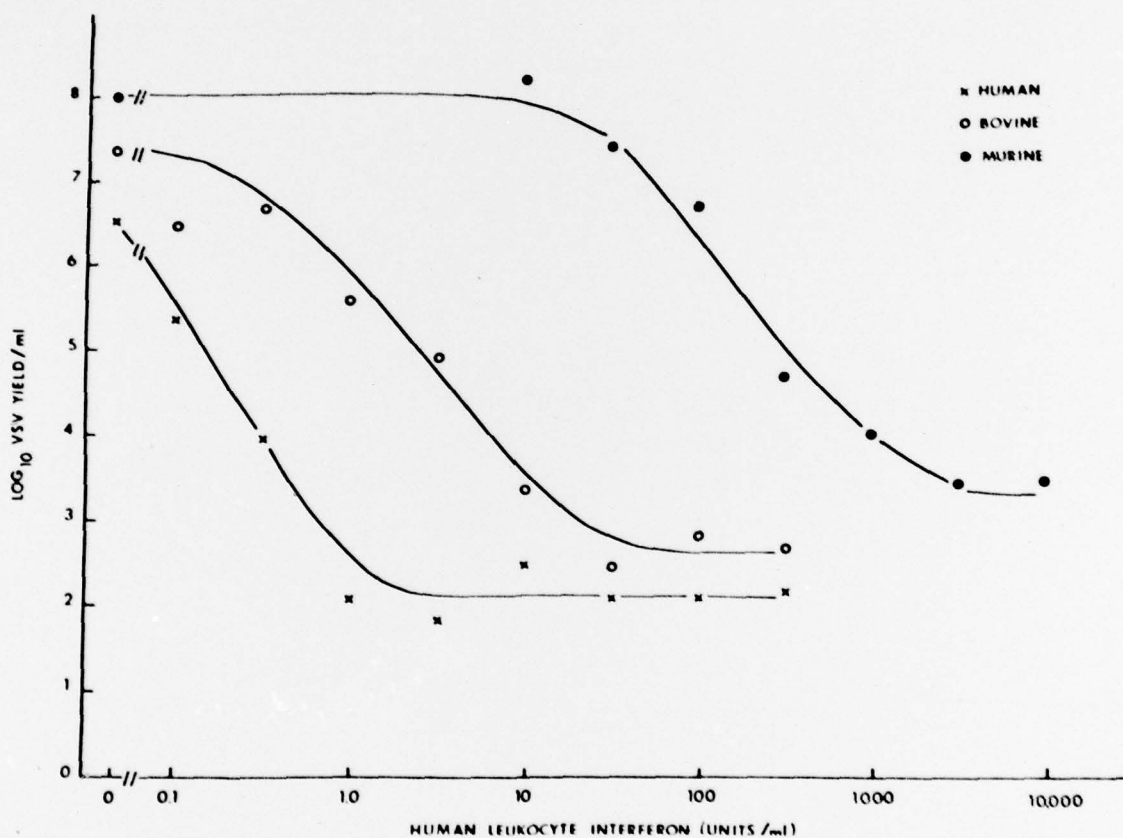
Figure 1

Dose-response study of the antiviral activity of human leukocyte
interferon on human, bovine, and murine cell cultures. Groups
of cell cultures were incubated for 18 hrs with the designated
quantity of interferon (units/ml) after which the cultures were
infected with vesicular stomatitis virus (VSV) at a MOI=1. Ten
hours later the supernatants were collected after a single cycle
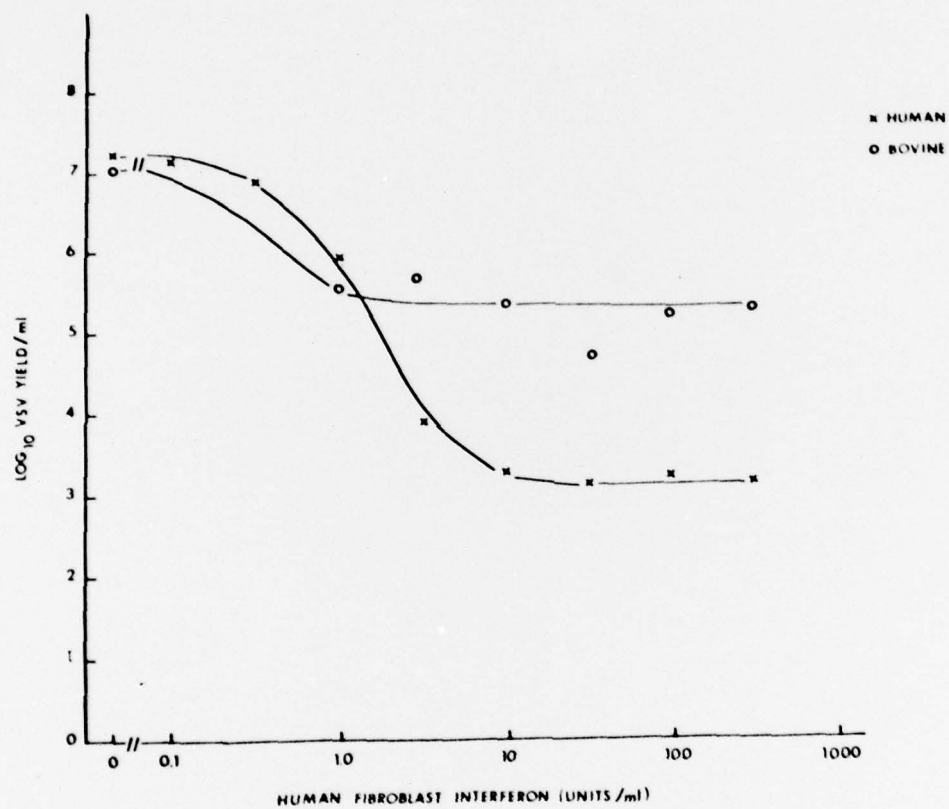of VSV multiplication and titrated by plaquing the VSV on vero
cells.

Figure 2

Dose-response study of the antiviral activity of human fibro-
blast interferon on human and bovine cells.

to a distinct class of cellular receptors. We have pre-
liminary evidence which indicates that cultured bovine kid-
ney cells synthesize two species of interferons, the minor
one being leukocyte-like, and the major one being a bovine
"fibroblast". These two distinct bovine interferons are ac-
tive on human cultures, and the nature of antiviral activities
expressed by them resembles the actions of the corresponding
human interferons on bovine cells i.e., the bovine fibroblast
interferon only induces a partial antiviral state on human
cells, and the bovine leukocyte-like interferon induces a
full state of resistance.

This past year, we have developed an extremely sensitive
quantitative neutralization assay which uses diminishing con-
centrations of specific anti-interferon serum which are re-
acted with a constant concentration (20 units) of an interferon
to determine the degree of neutralization obtained for a given
interferon activity. The resulting neutralization curves
reveal the relative amount of interferon required for the
particular biological activity in question, as well as the
presence of antigenically distinct interferon subspecies not
neutralized in a manner similar to other activities. A
difference in the slopes of the neutralization curves for
two different biological activities of an interferon would
possibly mean that the two activities are mediated through
different molecular regions of the same molecule. The ability
of an antiserum to neutralize one activity, but not another,
would suggest either that the activity that is unaffected by
the antiserum is due to another factor or possibly that the
molecular site responsible for this activity resided in a
molecular region not blocked or distorted by the antibody.

The neutralization curves for human leukocyte interferon
obtained by reacting varying dilutions of anti-leukocyte inter-
feron serum and then assaying for the residual antiviral
activity on bovine and two human fibroblast cell strains,
FS-4 and GM-258, are shown in Figure 3. It is important to
realize that GM-258 cells are trisomic for chromosome 21, and
that this chromosome contains the gene for antiviral resis-
tance. Consequently, cells trisomic for this chromosome have
been found to be 8-10 times more sensitive to interferon than
normal diploid human fibroblasts (FS-4) (15,16).

The biphasic neutralization curve obtained on the human
GM-258 cells was determined to be due to a small amount of
human fibroblast interferon present in the leukocyte inter-
feron. When anti-human fibroblast serum was added to the
reaction mixtures prior to incubation with the GM-258 cultures,
the plateau of protection was abolished and the resulting
neutralization curve resembled the sigmoidal curves obtained
for human FS-4 and bovine cells (results not shown). Since

Figure 3

Anti-human leukocyte interferon serum neutralization of the anti-
viral of human leukocyte interferon activity on bovine kidney
(MDBK), human diploid (FS-4) and trisomic (GM-258) fibroblasts.
Serial two-fold dilutions of antiserum were incubated with equal
volumes of human leukocyte interferon (20 units/ml) for 1 hr at
37°C. The mixtures were then assayed for antiviral activity
on the respective cells as in the dose-response studies. The
degree of interferon neutralization is expressed as $\log_{10}$ in-
hibition of VSV yield.

interferon preparations frequently consist of two antigenically distinct interferon species (17,18), this neutralization assay can be used to identify antigenically distinct interferon species in various interferon preparations.

Considerably more antibody (8-10 times) was required to neutralize the same concentration of human leukocyte interferon on the trisomic human GM-258 cells than was needed to neutralize the antiviral activity on human diploid (FS-4) and bovine cultures. Thus, the amount of antibody required to neutralize a given quantity of interferon is directly proportional to the activity expressed i.e., the more active the interferon, proportionately more antibody is required to neutralize that activity. Since the same leukocyte interferon was 8-10 times more active on human GM-258 cultures, than on either bovine cells or human diploid cells (FS-4), 8 times more antibody was required to neutralize its activity on human GM-258 cells.

The same relationship between activity and antibody-neutralizing titer was found when human fibroblast interferon was reacted with diminishing concentrations of specific anti-human fibroblast serum before being assayed on human diploid (FS-4) and trisomic (GM-258) fibroblast cultures (Figure 4). The resulting neutralization curves of human fibroblast interferon on both human fibroblast cell types are similar, whereas the pattern of neutralization on bovine cells is radically different and reflects the degree of sensitivity of the bovine cultures to human fibroblast interferon observed in figure 2.

II. In Vitro and In Vivo Production of Murine Interferons

We have recently become interested in murine interferons for the following reason: distinct murine interferons have now been identified which correspond to the three types of human interferons. Therefore, through the use of animal models it may prove possible to study the possible immunoregulatory properties of the various interferon classes in vivo. The use of animal models will also enable us to establish the best dose, route of administration, and the relative efficacy of each interferon in the treatment of different viral diseases.

A. In Vitro, Interferon Production

Confluent cultures of murine LPA cells were induced with varying concentrations of the synthetic polyribonucleotide polyinosinic-polycytidylic acid (Poly I-Poly C) and DEAE-dextran for a period of six hours or with Newcastle disease virus (NDV) at different multiplicities of infection (MOI) for 1 hour. Following the designated incubation periods, the inducing agents were removed, the cultures washed, and reincubated overnite with fresh medium. The culture supernatants were then collected and acidified to pH 2.0 to destroy virus and 5 days later the samples were returned to neutrality and assayed
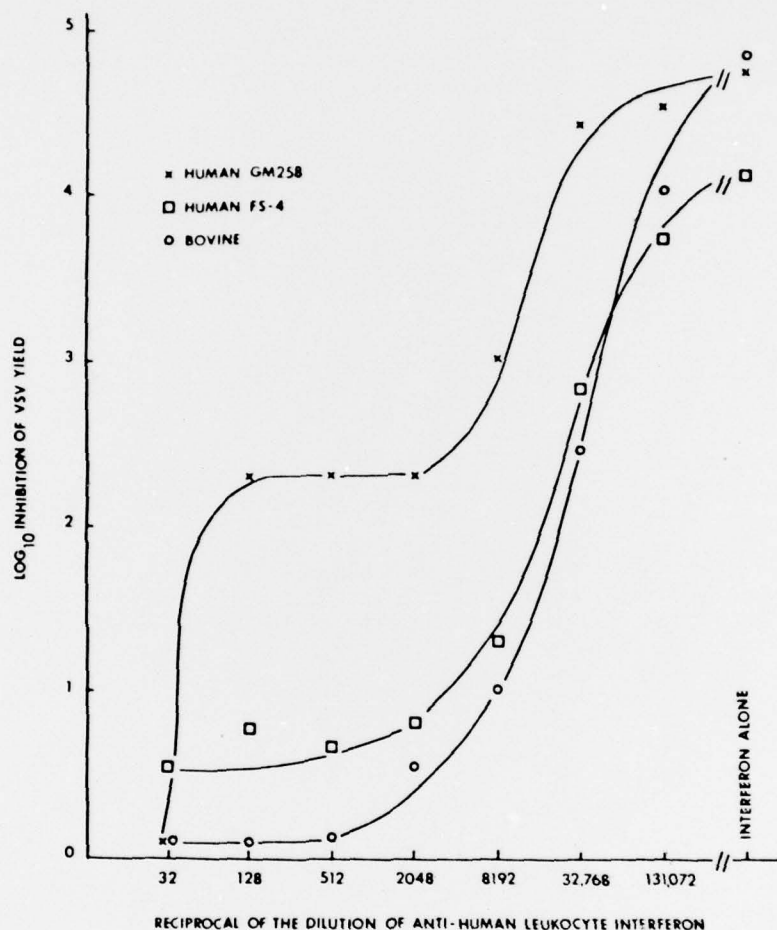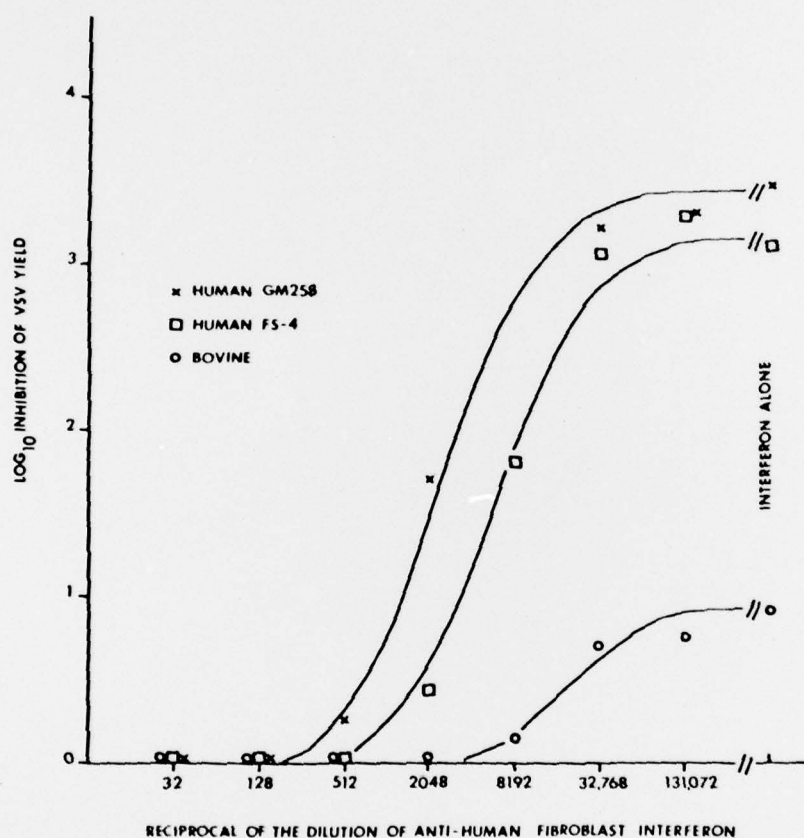
Figure 4

Anti-human fibroblast interferon serum neutralization of the
antiviral activity of human fibroblast interferon on bovine
kidney (MDBK), human diploid (FS-4), and trisomic (GM-258) fibro-
blast cell cultures.

on murine and human cells. The data presented in table 1 are
the optimum concentrations of either poly (I)-poly (C) or NDV
for interferon induction in our strain of L cells. The ability
of the virus-induced interferon to cross over on human cells is
due to the presence of a subspecies of interferon in these
preparations which is antigenically related to human leukocyte
interferon (19).

Table 1

IN VITRO MURINE INTERFERON INDUCTION

A. Type 1 Interferon

Cell	Inducing Agent	Interferon titer on	
		Murine	Human
L cells (LPA)	20µgs Poly(I)·Poly(C) +400µgs DEAE dextran/ml	8,192	<4
	NDV (MOI 2)	24,576	12
	NDV (MOI 20)	16,384	8

B. Type II Interferon

Spleen cells/ml.	PHA/ml	Interferon titer at hour after stimulation		
		24	48	72
$2x10^6$	none	<4	<4	<4
$2x10^6$	4µgs	12	6	24
$4x10^6$	none	<4	<4	<4
$4x10^6$	4µgs	24	32	32
$8x10^6$	none	<4	<4	<4
$8x10^6$	4µgs	48	96	96

Murine type II interferon is being induced by incubating
spleen cell cultures with the T-cell mitogen, phytohaemagglu-
tinin (PHA). Varying concentrations of spleen cells, obtained
by teasing cells loose from whole spleens, were incubated in
RPMI medium containing 4µgs PHA, and the culture fluids
collected from different groups of cultures at 24, 48, and 72 hours.
In Table 1, it is shown that the larger numbers of cells re-
sults in higher levels of interferon, with peak levels being
synthesized between 24 and 48 hrs after exposure to the PHA.
This spleen cell interferon was characterized as immune inter-
feron by its acid lability (type I interferons are stable in

acid) and the inability of rabbit anti-L cell interferon serum
to neutralize its antiviral activity (Table 2).

Table 2

INITIAL CHARACTERIZATION OF VARIOUS MURINE INTERFERON PREPARATIONS

| Interferon Source | Inducing Agent | Original Titer | Interferon activity after | | Neutralization titer[c] of anti-L[d] interferon serum on antiviral activity |
			pH 2.0[a]	Δ 1/56°C[b]	
L cells	Poly(I)·Poly(C)	8,192	8,192	n.d.	1000
Spleen cells	PHA	64	<8	n.d.	<20
Serum	Poly(I)·Poly(C)	8,192	8,192	16	2000
	Endotoxin	1,024	200	32	600
Serum from BCG infected mice	Old tuberculin	192	<8	48	<20

a Samples dialyzed against Sorensens HCL-Glycine buffer, pH 2.0, for 5 days
b Samples heated at 56°C for 30 minutes
c Reciprocal of the dilution of antiserum which can neutralize 20 units of interferon in an equal volume
d Rabbit anti-mouse L interferon serum

B.   In Vivo Interferon Induction

The in vivo induction of murine type II interferon was
performed by injecting BCG intravenously into mice and
challenging these animals with 10 µgs of old tuberculin (OT)
at weekly intervals thereafter. Two hours after injection of
the OT, the sera were collected and assayed for interferon (Table 3).
Mice infected with 10^8 BCG produced peak interferon levels in
response to OT at 2 weeks of infection, whereas animals infected
with 10^7 BCG produced peak levels at 3 weeks. The yields of
immune interferon obtained by this method (7) approach the levels
of type I interferon induced by intravenous injection of poly (I) -
poly (C), and far exceed the levels of type I interferon in-
duced by endotoxin (Table 3). As with in vitro generated
immune interferon, the interferon obtained by injecting OT
into BCG-infected animals was demonstrated to be immune inter-

feron by its acid lability, plus its stability at 56°C/30 minutes, and the inability of anti-L cell interferon serum to neutralize its antiviral activity (Table 2). These pilot studies demonstrate that we can induce both type I and type II murine interferons in vivo and in vitro.

Table 3

IN VIVO MURINE INTERFERON INDUCTION

A. Type I Interferon

Interferon inducing agent	Interferon titer at hour after stimulation						
	1	2	3	4	6	8	24
20µgs Poly(I)·Poly(C)	192	12,288	2,048	4,096	254	192	8
25µgs Endotoxin	64	374	96	64	64	16	4

B. Type II Interferon

Time of BCG infection	Number of BCG administered	Interferon inducing agent	Serum interferon titer + 2 hrs post OT challenge
1 week	10^7	10 mg OT	–
	10^8	"	16
2 weeks	10^7	"	512
	10^8	"	3072
3 weeks	10^7	"	4096
	10^8	"	768

C. Anti-Chlamydial Action of Interferons

Interferon preparations and interferon inducing agents have been shown to inhibit the intracellular multiplication of organisms other than viruses (4,5). I have always been fascinated by the unique replication cycle which chylamydia undergo within the phagocytic vacuole of host cells, including professional phagocytes such as macrophages. In the last year, I have had the opportunity to establish a collaborative arrangement with Dr. Gerald Byrne and his graduate student Constance Rothermel at the Cornell Medical School in New York City. These individuals have a murine

L cell system in which they propagate and quantitate chlamydia.
Together, we have undertaken a series of studies to determine
if interferon inhibits chlamydial growth, and if so, what
stage of the chlamydial multiplication cycle is affected. The
aim is to determine whether the cellular mechanism which in-
hibits chlamydial multiplication is the same as that which
inhibits viral multiplication.

The results of these joint studies thus far have estab-
lished that the growth of a strain of the human pathogen
lymphogranuloma venerum (LGV) in murine L cells is inhibited
by pretreating the cells with 300 murine interferon units.
Studies with inhibitors of RNA and protein synthesis clearly
established the need for protein synthesis by the host cell
for the interferon-induced anti-chlamydial resistance. Other
studies have established that the uptake of the chlamydia by
the cells is not inhibited by interferon. Preliminary evidence
indicates that the cellular mechanism which inhibits multipli-
cation of this strain of chlamydia may differ from that which
inhibits virus multiplication. Currently, we are planning to
examine the effect of murine type II interferon on the
multiplication of various chalmydia strains. Eventually,
we plan to study the effect of the various human interferons
on the intracellular multiplication of a number of human patho-
genic strains of C. trachomatis in human cells.

Discussion

The immediate goal of our studies is the systematic
analysis of the physical and biological properties of dif-
ferent classes of interferons. The combined results of the
log dose-response studies and the neutralization assays for
human leukocyte and fibroblast interferons clearly demon-
strate that these two human interferons differ in their anti-
viral actions on cells from other species. Human leukocyte
interferon crosses the species barrier and exerts its anti-
viral activity on cells of heterologous species in the same
manner as on human cells. The similarities in the antibody
neutralization curves for the various antiviral activities of
human leukocyte interferon suggest that the expression of the
various antiviral activities by this interferon is mediated
through the same molecular region of the interferon molecule.
The differences in sensitivities of the different cells to
leukocyte interferon probably reflect differences in affini-
ties of the interferon for cellular receptors of each different
species. The restricted capacity of human fibroblast inter-
feron to induce antiviral resistance in bovine cells and our
recent finding that one of the two distinct bovine interferons
induces a partial state of antiviral resistance on human cul-
tures indicates that there is a recognition of the heterologous
cellular receptors by these two 'fibroblast' interferons. How-
ever, these interferons fail to completely trigger the events
leading to the full development of an antiviral state on heterolo-
gous cells. This suggests that there may be a two step mech-
anism involved in the development of cellular antiviral resis-
tance: an initial recognition and binding of the interferons
to the cellular receptor, and a subsequent triggering of the
antiviral state. The degree of cellular resistance developed
is probably determined by the relative molecular interaction
between the interferon and cellular receptor.

The ultimate aim of our research is to determine the
function(s) of each interferon in vivo. Our studies, as well
as those of others, have clearly established that a single
species can synthesize different interferon molecules which
appear capable of mediating a number of biological effects
which include immunomodulatory functions and the ability to
inhibit the multiplication of obligate intracellular parasites
other than viruses. It now appears that three classes of
murine and bovine interferons exist which correlate with the
three classes of human interferon and antigenic homologies also
exist between certain interferons of each species. The finding
that human leukocyte and fibroblast interferons differ in their
abilities to mediate protection on cells of different species
indicates that the molecular interactions with cellular receptors
differ and this strongly suggests that perhaps each interferon
has evolved to perform specific in vivo functions. My decision
to move to the Trudeau Institute during the past year, was

based on my desire to use my expertize in interferons to study the role of these molecules in cellular immunity. The facilities at the Trudeau Institute will allow large scale production of murine immune interferon both in vitro and in vivo. The availability of different in vitro and experimental animal model systems being used to study cell-mediated immunity will enable us to establish whether interferons are elaborated during the course of specific immune reactions and to determine the roles interferons may play in in vivo protective functions. If an interferon alters a response in vivo, then, in vitro systems will enable the identification of the cellular component affected. Thus, through the integrated use of in vivo models and corresponding in vitro analysis, it will be possible to determine if interferons function in the regulation of cell-mediated immunity.

Conclusions

Interferons have proven effective in the treatment of a number of viral and malignant diseases. The concise and systematic characterization of each human interferon species as to its biological, physicochemical, and antigenic properties should offer valuable insights into the possible biological function of each interferon in the host's defensive mechanism against disease. These studies should also help to assess the relative clinical potential of each human interferon.

Significant Accomplishments

1. The isolation of a murine interferon subspecies, by means of antibody affinity using immobilized anti-human leukocyte interferon serum, which is antigenically related to human leukocyte interferon and is responsible for all the heterologous human antiviral activity of the unfractionated murine interferon preparation. We have also identified a bovine interferon which possesses antigenic homology with human leukocyte interferon.

2. The development of a quantitative antibody neutralization assay which is being used to determine both the degree of neutralization and whether the resulting neutralization curve for a particular interferon activity differs from those of other interferon activities. In addition this assay can detect the presence of small quantities of antigenically distinct interferons which would not normally be detected by conventional antibody neutralization assays.

3. The large scale production of human, bovine, and murine interferons. These interferons will enable the continuation of our physical and functional analysis of interferons and be used for immunization purposes.

4. In a collaborative study with investigators at Cornell Medical School we have found that interferon treatment of cells in culture inhibits a strain of chlamydia (LGV) and that the cellular mechanism which inhibits this obligate intracellular parasite may differ from the one which inhibits virus multiplication.

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Interferons are glycoproteins which induce states of cellular resistance to viral multiplication. It is now evident that interferons are capable of other biological actions such as the inhibition of cell division, macrophage activation, and suppression of intracellular multiplication of organisms other than virus. Inorder to determine if the various biological activities of each human interferon are mediated through common or distinct mechanisms, an inter-

20. Abstract - continued

feron neutralization assay was developed which uses diminishing concentrations of specific anti-interferon serum to access the degree of neutralization of each biological activity. The resulting neutralization curves reflect the relative quantity of interferon required for the expression of each activity, allow the comparison of slopes, and enable the detection of antigenically distinct interferon subspecies present in the interferon preparation. The antibody neutralization curves for human leukocyte interferon antiviral activities on homologous human and heterologous bovine cultures were similar. The results of similar studies using specific antibody to neutralize human fibroblast interferon antiviral activities on human and bovine cells revealed that not only was fibroblast interferon less active on bovine cells, but the slope of the neutralization curve on bovine cells differed from that on human cultures. This finding suggests that the mode of action of human fibroblast interferon on bovine cells differs from that on human cells.

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